

REMARKS

Claims 1-23 are active in the present application.

Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. Sequence Identifiers (SEQ ID NO:) have been added to the specification. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the Sequence Listing. Support for all of the sequences listed in the Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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IN THE SPECIFICATION

Page 4, second paragraph, delete in its entirety and insert therefor the following new paragraph.

--More particularly, in its first aspect, the invention provides a nucleic acid characterized in that it has a transcriptional promoter activity and in that it comprises:

- (a) all or a portion of sequence SEQ ID [n° 2] NO:3 or a variant thereof; or
- (b) a sequence hybridising with all or part of the complementary strand of sequence SEQ ID [n° 2] NO:3--

Page 4, paragraph beginning on line 13, delete in its entirety and insert therefor the following new paragraph.

--More preferably, the nucleic acid of the invention comprises all or a portion of sequence SEQ ID [n° 2] NO:3--

Page 4, paragraph beginning on line 29 and ending on page 5, line 7, delete in its entirety and insert therefor the following new paragraph.

--The term "portion" or "fragment" of nucleic acid means any nucleic acid comprising at least a portion of the sequence under consideration (for example sequence SEQ ID [n° 2]) NO:3 and which retains a transcriptional promoter activity. The sequence portion advantageously contains at least 50 bp, more preferably at least 100 bp. These "portions" can readily be generated using conventional molecular biological techniques, either by enzymatic

cleavage and digestion from the fragments describes, or by synthesis using nucleic acid synthesisers.--

Page 5, paragraph beginning at line 4, delete in its entirety and insert therefor the following new paragraph.

--The term "hybridising" as used in the present invention means any hybridisation under normal conditions, which may be stringent or non stringent, as defined below. An example of stringent hybridisation conditions is: Hybridisation at 42°C, 50% formamide, 5 X SSC, 1 X Denhardt; Wash at 65°C in 0.1 X SSC, 0.1% SDS. Non stringent conditions are: Hybridisation at 37°C, 40% formamide, 5 X SSC, 1X Denhardt; Wash at 50°C in 1 X SSC, 0.1% SDS. Stringent conditions are particularly suitable when the nucleic acids are present in small quantities and/or are in purified form. Non stringent conditions are more suitable when the nucleic acid present in large quantities and are significantly represented in the sample. Advantageously, "hybridising" sequences are sequences which hybridise under stringent conditions, and which thus have a high degree of structural homology with the sequence under consideration (for example SEQ ID [n°] NO:2) or its fragments. Further, hybridising sequences can include a region enabling hybridisation and a contiguous region which is not hybridising, but corresponding to flanking regions.--

Page 6, paragraph beginning at line 17, delete in its entirety and insert therefor the following new paragraph.

--To this end, the inventors have also demonstrated the existence in the identified gene of such a secretion signal, which is particularly active in bacteria from the genus *Clostridium*. This signal is represented by residues 268-357 in sequence SEQ ID [n°] NO: 1, and separately in SEQ ID [n° 3] NO:4. This signal, or any variant or active fragment thereof, constitutes an advantageous embodiment of the invention.--

Page 14, paragraph beginning at line 29, delete in its entirety and insert therefor the following new paragraph.

--Figure 8: Hydrophobicity profile for beta 2 toxin (SEQ ID NO:2).--

Page 15, paragraph beginning at line 1, delete in its entirety and insert therefor the following new paragraph.

--SEQ ID [n°] NO:1: Gene sequence for beta 2 toxin of type C *Clostridium perfringens*.

SEQ ID [n° 2] NO:3: Sequence for beta 2 toxin gene promoter of type C *Clostridium perfringens*.

SEQ ID [n° 3] NO:4: Sequence for secretion signal of beta 2 toxin gene of type C *Clostridium perfringens*.

SEQ ID [n° 4] NO:6: Sequence of primer P318.

SEQ ID [n° 5] NO:7: Sequence of primer P292.--

Page 16, lines 29 and 30, delete in their entirety and insert therefor:

--P318: 5'-GAAATGTTACAACGTATTAAATATCGTAG-3' (SEQ ID [n° 4] NO:6)

P292: 5'-TCAAGTTGTACATGGGATGATG-3' (SEQ ID [n° 5] NO:7)--

Page 17, paragraph beginning at line 8, delete in its entirety and insert therefor the following new paragraph:

--The sequence obtained in Example A (SEQ ID [n°] NO:1) comprises an open reading frame coding for the mature beta 2 toxin (residues 358 to 1122), and regulating or addressing regions located at the 5' and 3' end. The promoter region in the gene of the beta 2 toxin of type C *Clostridium perfringens* can be pinpointed on this sequence as comprising residues 1 to 267. This promoter sequence in the beta 2 toxin gene of type C *Clostridium*

perfringens is shown separately in sequence SEQ ID [n° 2] NO:3. This sequence includes a consensus ribosome binding site (GGGGGG) located 7 nucleotides upstream of the start codon ATG, i.e., at positions 255-260 in sequence SEQ ID [n° 2] NO:3--

Page 17, paragraph beginning at line 17, delete in its entirety and insert therefor the following new paragraph.

This region, or any fragment or variation thereof, can be isolated from samples of *Clostridium* nucleic acids using suitable probes (for example corresponding to sequence SEQ ID [n° 2] NO:3 or a fragment thereof) or by chemical synthesis, or by enzymatic digestion from the plasmids of the invention, in particular the plasmid pMRP268, deposited on 8th August 1997 at the Collection of the Institut Pasteur (CNCM: Collection Nationale de Cultures de Microorganismes), accession number I-1911.--

Page 18, paragraph beginning at line 5, delete in its entirety and insert therefor the following new paragraph.

--In addition to an open reading frame and transcription regulation regions (Example B), the sequence obtained in Example A (SEQ ID [n°] NO:1) also comprises addressing signals enabling a protein or peptide to be directed during synthesis towards the host cell secretion routes. The addressing region (secretion signal peptide) of the beta 2 toxin gene of type C *Clostridium perfringens* can be seen in sequence SEQ ID [n°] NO:1 as including residues 268 to 357. This signal peptide sequence in sequence SEQ ID [n° 3] NO:4. This region codes for 30 amino acids, comprising a hydrophobic region (residues 6-26), probably forming a transmembrane domain, bordered by charged amino acids (Lys2, Lys3, Lys7 and Lys27). Further, the junction region between this signal sequence and the mature protein (Ala30-Lys31) corresponds to the (Ala-X) cleavage site of the major portion of bacterial signal peptidases.--

Page 18, paragraph beginning at line 18, delete in its entirety and insert therefor the following new paragraph.

--This region, or any fragment or variant thereof, can be isolated from samples of *Clostridium* nucleic acids using suitable probes (for example corresponding to sequence SEQ ID [n° 3] NO:4 or a fragment thereof) or by chemical synthesis, or by enzymatic digestion from plasmids of the invention, in particular plasmid pMRP268, deposited on 8th August 1997 in the Institut Pasteur collection (CNCM), accession number I-1911.--

IN THE CLAIMS

--1. (Amended) A nucleic acid characterized in that it has a transcriptional promoter activity and in that it comprises:

- (a) all or a portion of sequence SEQ ID [n° 2] NO:3 or a variant thereof; or
- (b) a sequence hybridising with all or part of the complementary strand of sequence SEQ ID [n° 2] NO:3.

2. (Amended) A nucleic acid according to claim 1, characterized in that it comprises all or a portion of sequence SEQ ID [n° 2] NO:3.

19. (Amended) A nucleic acid comprising all or a portion of sequence SEQ ID [n° 3] NO:4--

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